

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
)
V. Sandig, et al.) Group Art Unit: 1633
)
Application No.: 10/578,043) Examiner: Leavitt, M.G.
)
Filed: January 9, 2008) Confirmation No.: 5415
)
For: Immortalized Avian Cell Lines for Virus)
Production)
)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, INGO JORDAN, hereby declare:

1. I hold a Ph.D. degree in biochemistry and retrovirology and consider myself one of skill in the art of virology.
2. I have published 13 articles indexed in the "PubMed.gov" NCBI library.
3. I have reviewed the claims of U.S. Application No. 10/578,043, and I have been informed that these claims are rejected on the basis of obviousness, that one of skill in the art would have recognized that the results of the combination of E1A and E1B genes would have yielded the predictable results of inducing cell proliferation by disrupting Rb/E2F complexes and inactivating p53 in avian cells, purely due to their known interaction and activity in human and mammalian cells.

4. I disagree with the Office Action's assertion. I am of the view that the immortalization of avian cells with E1A and E1B 55K from mastadenoviruses is an unexpected result.

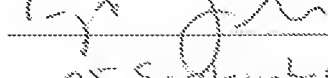
5. The inventors have observed that human adenoviruses cannot replicate in avian cells. However, the inventors noticed that human adenoviruses can successfully enter avian cells. Thus, the lack of replication is not due to the fact that said adenoviruses do not find the correct receptors for viral entry. It is predicted that the viral regulatory pathways within the avian cell, which include E1A and E1B 55K, appear to be dysfunctional when compared to human and mammalian cells.

6. It is clear that human p53 and avian p53 share very few conserved amino acid sequences. I have looked at the human and chicken p53 protein sequences. The sequence identity between human p53 (amino acid sequence according to *Annex I* enclosed herewith) and chicken p53 (amino acid sequence according to *Annex II* enclosed herewith) is only 53.7% (see sequence alignment between the whole length human p53 and chicken p53 of *Annex III* enclosed herewith). Moreover, the sequence identity between the N-terminal transactivation domain (amino acids 1 to 58) of human p53 which is bound by E1B 55K of human adenovirus and rendered inactive and chicken p53 is only 29.9% (see sequence alignment between the N-terminal transactivation domains of human p53 and chicken p53 of *Annex IV* enclosed herewith).

7. Due to this difference in amino acid sequence, the binding activity of avian p53 cannot be predicted by human p53 results. Thus, it is unknown whether the E1A and E1B proteins will have the same immortalization effect, or any effect, on avian cells.

8. Considering the above, the skilled person in the art would have not expected that E1A and E1B 55K from mastadenoviruses would have the same activity in avian cells than in mammalian cells, i.e. that E1A and E1B 55K from mastadenoviruses can also affect the avian homologues of Rb, E2F and p53.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. All of the statements I made herein are made of my own volition. I understand that willful false statements may subject me to fines, imprisonment or both, pursuant to Section 1001 of Title 18 of the United States Code.

Signed: 
Date: 05 September 2011

Amex I

Protein

Translations of Life

Display Settings: GenPept

P53 [Homo sapiens]

GenBank BAC16799.1

EMBL GenBank

GenBank

LOCUS BAC16799 393 aa linear PRI 01-APR-2003
 DEFINITION P53 [Homo sapiens].
 ACCESSION BAC16799
 VERSION BAC16799.1 GI:23491729
 DBSOURCE accession AB082923.1
 KEYWORDS
 SOURCE Homo sapiens (human)
 ORGANISM Homo sapiens
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
 Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
 Catarrhini; Hominidae; Homo.
 REFERENCE 1
 AUTHORS Azuma, K., Shichijo, S., Maeda, Y., Nakatsura, T., Nonaka, Y., Fujii, T.,
 Koike, K. and Itch, K.
 TITLE Mutated p53 gene encodes a nonmutated epitope recognized by
 HLA-B*4601-restricted and tumor cell-reactive CTLs at tumor site
 JOURNAL Cancer Res 63 (4), 854-858 (2003)
 PUBMED 12591737
 REFERENCE 2 (residues 1 to 393)
 AUTHORS Shichijo, S. and Itou, K.
 TITLE Direct Submission
 JOURNAL Submitted (26-MAY-2002) Shigeki Shichijo, Kurume Univ. School of
 Med., Dep. Immunol., 67-Asahi-machi, Kurume, Fukuoka 830-0011,
 Japan (E-mail: shichijomed.kurume-u.ac.jp, Tel:81-942-31-7551,
 Fax:81-942-31-7699)
 FEATURES
 source Location/Qualifiers
 1..393
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /clone="7P"
 /cell_line="SW620"
 /cell_type="colon cancer"
 Protein 1..393
 /product="P53"
 Region 5..29
 /region_name="P53_TAD"
 /note="P53 transactivation motif; pfam08563"
 /db_xref="CDD:149567"
 Region 109..295
 /region_name="P53"
 /note="P53 DNA-binding domain; cd08367"
 /db_xref="CDD:176262"
 Site order(176,179,236,242)
 /site_type="other"
 /note="zinc binding site"
 /db_xref="CDD:176267"
 Site order(177..179,181)
 /site_type="other"
 /note="dimerization site"
 /db_xref="CDD:176262"
 Site order(239,241,248,273,275..277,280)
 /site_type="DNA binding"
 /db_xref="CDD:176262"
 Region 319..389
 /region_name="P53_tetramer"
 /note="P53 tetramerisation motif; pfam07710"
 /db_xref="CDD:149327"
 CDS 1..393
 /gene="p53"
 /coded_by="AB082923.1:64..1245"
 ORIGIN
 1 mscpgcdpav applsgetts dlwklipenn vlsplpsqam ddlnlapddi eqwftedgpp
 61 deaprppeaa prvapapaap tpakgapaps wplsssvpsq ktyggsgyfr lgflhshtak
 121 svctryspal nkmfcqlakt cpvqlwvdst ppgptrvram alykqsgbms evvrrephba
 181 roudscglap pgnlirvegn lrveyldden ttrhsrvrpy eppevgadct tihynyscns
 241 scnggmwarp iltitileda sgallgrnaf evtvccapgr arrteeenir kkgaphelp
 301 pgekkvrafn ntssspqphk xpldgyftr qirgrxkfw ireinealel kdagapkegs
 361 gsrashshlk ekkqgsrakh kklafktegp dsd

//

Annex II

Protein

Translations of Life

Display Settings: GenPept

RecName: Full=Cellular tumor antigen p53; AltName: Full=Tumor suppressor p53

Swiss-Prot P10360.1

FASTA Graphics

22.12

LOCUS P53_CHICK 367 aa linear VRT 30-NOV-2010
 DEFINITION RecName: Full=Cellular tumor antigen p53; AltName: Full=Tumor suppressor p53.
 ACCESSION P10360
 VERSION P10360.1 GI:129366
 DBSOURCE UniProtKB: locus P53_CHICK, accession [P10360](#), class: standard, created Jul 1, 1989, sequence updated: Jul 1, 1999, annotation updated: Nov 30, 2010.
 xrefs: [K13027.1](#), [CAA31456.1](#), [S02193](#), [NP_090595.1](#)
 xrefs (CCO-sequence databases): [IPT](#):[IP000585109](#), [UniGene](#):[Gga.706](#), [ProteinModelPortal](#):[P10360](#), [SMR](#):[P10360](#), [GeneID](#):[396200](#), [KEGG](#):[gga:396200](#), [CTD](#):[396200](#), [NOVERGEN](#):[XRG00520A](#), [GO](#):[GO:0001737](#), [GO](#):[GO:0005634](#), [GO](#):[GO:0003677](#), [GO](#):[GO:0001702](#), [GO](#):[GO:0006370](#), [GO](#):[GO:0006915](#), [GO](#):[GO:0007049](#), [GO](#):[GO:0001010](#), [GO](#):[GO:0005555](#), [GO](#):[GO:0003347](#), [GO](#):[GO:0005590](#), [InterPro](#):[IPR000867](#), [InterPro](#):[IPR012346](#), [InterPro](#):[IPR015511](#), [InterPro](#):[IPR011616](#), [InterPro](#):[IPR010991](#), [InterPro](#):[IPR002117](#), [Gene3D](#):[G3DSA:2.40.40.720](#), [Gene3D](#):[G3DSA:4.10.170.10](#), [PRINTS](#):[P00870](#), [Pfam](#):[PF00870](#), [Pfam](#):[PF07710](#), [PRINTS](#):[P00366](#), [RFPfam](#):[S5F49417](#), [SUFAM](#):[S5F47719](#), [PROSITE](#):[PS00348](#)
 KEYWORDS Activator; Apoptosis; Cell cycle; Cytoplasm; DNA-binding; Metal-binding; Nucleus; Phosphoprotein; Transcription; Transcription regulation; Tumor suppressor; Zinc.
 SOURCE *Gallus gallus* (chicken)
 ORGANISM *Gallus gallus*
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Archosauria; Dinosauria; Saurischia; Theropoda; Coelurosauria; Aves; Neognathae; Galliformes; Phasianidae; Phasianinae; Gallus.
 REFERENCE 1 (residues 1 to 367)
 AUTHORS Scoussi, T., Begue, A., Kress, M., Stehelin, D. and May, P.
 TITLE Nucleotide sequence of a cDNA encoding the chicken p53 nuclear oncoprotein
 JOURNAL Nucleic Acids Res. 16 (23): 11383 (1988)
 PUBMED 3060861
 REMARK NUCLEOTIDE SEQUENCE (MRNA), STRAIN-SPAFAS
 COMMENT On Apr 12, 2005 this sequence version replaced gi:186220.
 [FUNCTION] Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression (By similarity).
 [COFACTOR] Binds 1 zinc ion per subunit (By similarity).
 [SUBUNIT] Binds DNA as a homotetramer (By similarity).
 [SUBCELLULAR LOCATION] Cytoplasm (By similarity). Nucleus (By similarity).
 [SIMILARITY] Belongs to the p53 family.
 FEATURES
 Location/Qualifiers
 source 1..367
 /organism="Gallus gallus"
 /db_xref="taxon:9031"
 gene 1..367
 /gene="TP53"
 Protein 1..367
 /gene="TP53"
 /product="Cellular tumor antigen p53"
 /note="Tumor suppressor p53"
 /UniProtKB_evidence="Evidence at transcript level"
 Region 1..367
 /gene="TP53"
 /region_name="Mature chain"
 /experiment="experimental evidence, no additional details recorded"
 /note="Cellular tumor antigen p53. /FTId=PRO_0000185716."
 Region 1..30
 /gene="TP53"
 /region_name="Region of interest in the sequence"
 /experiment="experimental evidence, no additional details recorded"
 /note="Transcription activation (acidic)."
 Site 87..278
 /gene="TP53"
 /site_type="DNA binding"
 /evidence="non-experimental evidence, no additional details recorded"
 /note="By similarity."
 Region 94..275
 /gene="TP53"
 /region_name="P53"
 /note="P53 DNA-binding domain; cd00367"
 /db_xref="CDD:175252"
 order(161,164,224,228)
 /gene="TP53"

```

/site_type="other"
/note="zinc binding site"
/db_xref="CDD:178262"
Site
161
/gene="TP53"
/site_type="metal-binding"
/inference="non-experimental evidence, no additional
details recorded"
/note="Zinc (By similarity)."
```

Site

```

order(162..164,166)
/gene="TP53"
/site_type="other"
/note="dimerization site"
/db_xref="CDD:178262"
Site
164
/gene="TP53"
/site_type="metal-binding"
/inference="non-experimental evidence, no additional
details recorded"
/note="Zinc (By similarity)."
```

Site

```

324
/gene="TP53"
/site_type="metal-binding"
/inference="non-experimental evidence, no additional
details recorded"
/note="Zinc (By similarity)."
```

Site

```

order(228,227,234,259,261..263,265)
/gene="TP53"
/site_type="DNA binding"
/db_xref="CDD:178262"
Site
228
/gene="TP53"
/site_type="metal-binding"
/inference="non-experimental evidence, no additional
details recorded"
/note="Zinc (By similarity)."
```

Region

```

286..302
/gene="TP53"
/region_name="Short sequence motif of biological interest"
/inference="non-experimental evidence, no additional
details recorded"
/note="Bipartite nuclear localization signal (By
similarity)."
```

Region

```

300..342
/gene="TP53"
/region_name="P53 tetramer"
/note="P53 tetramerisation motif; pfam07710"
/db_xref="CDD:149007"
Region
308..319
/gene="TP53"
/region_name="Region of interest in the sequence"
/experiment="experimental evidence, no additional details
recorded"
/note="Oligomerization."
```

Region

```

322..333
/gene="TP53"
/region_name="Short sequence motif of biological interest"
/inference="non-experimental evidence, no additional
details recorded"
/note="Nuclear export signal (By similarity)."
```

Region

```

347..364
/gene="TP53"
/region_name="Region of interest in the sequence"
/experiment="experimental evidence, no additional details
recorded"
/note="Basic (repression of DNA-binding)."
```

ORIGIN

```

1 masemepille ptevfmdlws nlpyssqqlp lpedlwnwqe isplepedpp gppppppipl
61 aaapppplnp ytppraasps vvpstedygg dkdfrvgive agtakavtct yspvlkvyc
121 rlaakpcpvy rygvapppgs slravavykk nehvaevrrr cphhercggg tdylapaghi
181 irvegnpgar yhdetckrh svvvpyppep vgedcttlyl nfwncsacwe gmmarpilti
241 lclepppgql lgrxcfevr caopgrdki eeeufikrgg aggvakrams pptaapeppk
301 krvinpdnei tyiqvigrvv ywikeinca iqlaeggeap rpaqgrvvkv egpqpccgkh
361 ilqkged
```

//

Annex III

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EMBOSS Align Results

Needle Results	
Matrix	Blosum62
Open gap penalty	10.0
Gap extension penalty	0.5
Needle output	needle-20101223-0809184341.output
SUBMIT ANOTHER JOB	

```
#####
# Program: needle
# Runkdate: Thu 23 Dec 2010 08:09:16
# Commandline: needle
# [-asequence] /ebi/extserv/old-work/needle-20101223-0809184341.input.1
# [-bsequence] /ebi/extserv/old-work/needle-20101223-0809184341.input.2
# -outfile /ebi/extserv/old-work/needle-20101223-0809184341.output
# -gapopen 10.0
# -gapextend 0.5
# -datafile EMBLCSUM62
# -spprotein1
# -spprotein2
# -auto
# Align_format: syspair
# Report_file: /ebi/extserv/old-work/needle-20101223-0809184341.output
#####
```

```
#####
#
# Aligned_sequences: 2
# 1: EMBOS_001
# 2: EMBOS_001
# Matrix: EMBLCSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 397
# Identity: 213/397 (53.7%)
# Similarity: 250/397 (63.5%)
# Gaps: 34/397 ( 8.6%)
# Score: 1002.5
#
#
#####
```

p53 chicken
p53 homo sapiens

EMBOSS_001	1 mae-emepillep---tevfmdlwsalpyseqqlplpedhenwqelsp--1	44
EMBOSS_001	1 neeqqsdpsaveppisqetfscldwkilpennvisplpqagmddmlspddi	50
EMBOSS_001	45 ep--sdppppppppplplaa--aappplnppppraasp-----vvps	54
EMBOSS_001	51 eqwftedpgqdesprnceaaprvaapapa-aptpaapapapewplsasvps	99
EMBOSS_001	85 tedygddfdfrvgfveagtaksvtcttyspvlhkvycriakpcpvqrvvgv	134
EMBOSS_001	100 gktyqgsygfrlgflhsgtaksvtcttyspalnkmfqqlaktcpvqlwvds	149
EMBOSS_001	135 apppgsslravavykksehvaevvrrcphhrcgggtgdlapaqhlirve	184
EMBOSS_001	150 tpppgtrvramaiykqsgmttevvrrcphhrc-sdsdglappghlirve	198
EMBOSS_001	185 gnpqaryhddettkrhsvvpyeppevggsdcttvlvnmcnsscmggnmr	214
EMBOSS_001	199 gnlrvsylddrnfchsvvpyeppevggsdcttihynmcnsscmggnmr	246
EMBOSS_001	235 rpiltaltlegpgqllgrrofevrvccpggrdrkieeenfrkrvgg----	280
EMBOSS_001	249 rpiltiitlledsgnllgrnsfevhvccpggrdrteeenlrkkgephhe	298
EMBOSS_001	281 -aggvakramsppteapeppkkrvlnpdneifylgvrgrrrycmkneine	329
EMBOSS_001	299 lppgstkralnsntssspgpkkrpl--dgyftlqirgrerfermfrelne	346

#-----
#-----

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EMBOSS Align Results

Needle Results	
Matrix	Blosum62
Open gap penalty	10.0
Gap extension penalty	0.5
Needle output	needle-20101223-1400596906.output

[SUBMIT ANOTHER JOB](#)

```
#####
# Program: needle
# Rundate: Thu 23 Dec 2010 14:01:00
# Commandline: needle
# [-asequence] /ebi/extserv/old-work/needle-20101223-1400596906.input.1
# [-bsequence] /ebi/extserv/old-work/needle-20101223-1400596906.input.2
# -outfile /ebi/extserv/old-work/needle-20101223-1400596906.output
# -gapopen 10.0
# -gapextend 0.5
# -datafile EBLOSUM62
# -sprotein1
# -sprotein2
# -auto
# Align_format: srspair
# Report_file: /ebi/extserv/old-work/needle-20101223-1400596906.output
#####
```

```
#####
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 67
# Identity:      20/67 (29.9%)
# Similarity:    27/67 (40.3%)
# Gaps:          18/67 (26.9%)
# Score: 64.0
#
#
#####
```

```
EMBOSS_001      1 meepqsdpsvepplsqetfslwklpennvlsplpsqamddmlspddi      50
                  |. :.:.:| | .|.|||.|||.|||.|||.|||.|||.|||.|||.
EMBOSS_001      1 mae-emepilep---tevfndlvsmlpyamqqlplpedhsnwqelsple"      45

EMBOSS_001      51 eqwftedp-----      58
                  ..|
EMBOSS_001      46 ----psdppppppppppl      58
```

```
#-----
#-----
```